ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

Association between Lung Function in Adults and Plasma DDT and DDE Levels: Results from the Canadian Health Measures Survey

Ming Ye, Jeremy Beach, Jonathan W. Martin, and Ambikaipakan Senthilselvan

http://dx.doi.org/10.1289/ehp.1408217

Received: 3 February 2014
Accepted: 22 December 2014
Advance Publication: 23 December 2014

ERRATUM: When this article was originally posted online as an Advance Publication, it included two incorrect units of measure: μg/g lipid (used throughout the text and in Table 2) and mg/g lipid (used in Table 3). The correct unit of measure for all these instances is ng/g lipid. The affected units of measure have been corrected throughout the text and tables.
The authors regret the errors.

Association between Lung Function in Adults and Plasma DDT and DDE Levels: Results from the Canadian Health Measures Survey

Ming Ye,[1] Jeremy Beach,[1, 2] Jonathan W. Martin,[3] and Ambikaipakan Senthilselvan[1]

[1]School of Public Health, University of Alberta, Edmonton, Alberta, Canada; [2]Division of Preventive Medicine, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; [3]Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada

Address correspondence to Ambikaipakan Senthilselvan, School of Public Health, University of Alberta, 3-276 Edmonton Heath Clinic Academy, 11405-87 Avenue, Edmonton, Alberta, T6G 1C9 Canada. Telephone: (780) 492-6505. Fax: (780) 492-0364. E-mail: sentil@ualberta.ca

Running title: Plasma DDT level and lung function

Acknowledgment: M.Y. is supported by Public Health and the Agricultural Rural Ecosystem (PHARE) Training Award, Canadian Institute of Health Research and Queen Elizabeth II Graduate Studentship, University of Alberta.

Disclaimer: The research and analysis are based on the data from Statistics Canada and opinions expressed herein do not represent the views of Statistics Canada.

Competing financial interests: The authors have no actual or potential competing financial interests.

Abstract

Background: Although DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] has been banned in many countries since the 1970s, it may still pose a risk to human respiratory health. In agriculture, DDT exposures have been associated with asthma and chronic bronchitis. However, little is known about the effect of DDT on lung function.

Objectives: We examined DDT/DDE concentrations in plasma and associations with lung function FVC (forced vital capacity), FEV_1 (forced expiratory volume in one second), FEV_1/FVC ratio, and $FEF_{25\%-75\%}$ (forced expiratory flow between 25% and 75% of FVC).

Methods: We used data on 1,696 participants aged 20-79 years from the Canadian Health Measures Survey (CHMS) and conducted multiple regression analysis to estimate associations between plasma *p,p'*-DDT/DDE and lung function.

Results: Almost all participants (over 99.0%) had detectable concentrations plasma *p,p'*-DDE, while only 10.0% had detectable *p,p'*-DDT. Participants with detectable *p,p'*-DDT had significantly lower mean FVC (diff=311mL; 95% CI: –492, –130; p=0.003) and FEV_1 (diff=232mL; 95% CI: –408, –55; p=0.015) than those without. A 100 ng/g lipid increase in plasma *p,p'*-DDE was associated with an 18.8mL decrease in mean FVC (95% CI: –29, –9) and an 11.8mL decrease in mean FEV_1 (95% CI: –21, –3). Neither exposure was associated with FEV_1/FVC ratio or $FEF_{25\%-75\%}$.

Conclusions: DDT exposures, which may have occurred decades ago, were still detectable among Canadians. Plasma DDT and DDE were negatively associated with lung function parameters. Additional research on the potential effects of DDT use on lung function is warranted.

Introduction

DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane], an organochlorine insecticide, was once widely used to control insects in agriculture (WHO 1979) as well as insect-transmitted diseases, such as malaria and typhus (Attaran and Maharaj 2000). DDT can naturally break down into DDE [1,1-bis-(4-chlorophenyl)-2,2-dichloroethene] and DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)ethane] through photolysis and microbial biodegradation (ATSDR 2002). In humans, DDT can be either oxidized or reduced by cytochrome P450 enzymes (CYP450) to form DDE or DDD (Chen et al. 2009). DDE can further undergo epoxidation and phase II metabolism and DDD can be further oxidized to DDA [2,2-bis(4-chlorophenyl)acetic acid] (Chen et al. 2009).

Both DDT and its breakdown products DDE and DDD are highly persistent in the environment. In the soil, DDT, DDE, and DDD can persist as long as 40 years or more (ATSDR 2002). In addition, DDT and its breakdown products are highly lipophilic and have the potential to bioaccumulate in fat tissue of exposed animals (Anderson 1985). DDT and its breakdown compounds can enter the human body through contaminated water, soil and food (Bayen et al. 2005; Perez-Maldonado et al. 2010). In humans, DDT and DDE have half-lives of 6 years, and up to 10 years, respectively (Longnecker 2005; Wolff et al. 2000). Previous studies have shown that DDT and/or DDE were detectable in almost all human blood and breast milk samples, which were collected mainly in the 1990s and 2010s from a number of global regions (Eskenazi et al. 2009; Perez-Maldonado et al. 2010; Smith 1999).

As a result of such environmental concerns, the use of DDT was greatly restricted or banned in most developed countries, including the US, Canada, and many European countries, in the 1970s.

A worldwide ban of DDT for agricultural use began in 2004 after the Stockholm Convention classified DDT as a persistent organic pollutant (POP) (UNEP 2010). Nevertheless, due to its ongoing use for disease vector control in some countries, high environmental persistence, and bioaccumulative properties, DDT and its breakdown compounds still pose potential risks to human health. Many adverse effects on human health DDT exposures have been associated with a variety of outcomes, including neurological (Keifer and Firestone 2007), immunological (Corsini et al. 2008), reproductive (Beard 2006), and respiratory outcomes (Ye et al. 2013), and some cancers (Beard 2006). In addition, there is experimental evidence that DDT has endocrine disrupting effects (De Coster and van Larebeke 2012).

A number of associations between respiratory health outcomes and DDT have been reported among agricultural pesticide applicators. For example, results from the US Agricultural Health Study demonstrated that adult-onset asthma was associated with exposures to DDT among farmers (Hoppin et al. 2008; Hoppin et al. 2009). The authors further suggested associations appeared to be more specific for atopic asthma among women (Hoppin et al. 2008). Another report based on the Agricultural Health Study suggested that duration of DDT exposure was significantly associated with chronic bronchitis (Hoppin et al. 2007). A retrospective cohort study of outdoor pesticide applicators in Australia also reported that asthma mortality was higher among workers who were occupationally exposed to insecticides, including DDT (Beard et al. 2003).

While there have been some studies of the effects of DDT exposure on respiratory diseases, few have focussed on its impact on lung function. In the current study, association of DDT and its metabolite DDE with lung function was estimated using data from the Canadian Health Measures Survey (CHMS).

Methods

Study population

From 2007 to 2009, Statistics Canada conducted the Canadian Health Measures Survey (CHMS, Cycle 1), a cross-sectional survey collecting baseline health information of Canadians (Statistics Canada 2011). In the current study, we used data on 1,696 participants aged 20-79 years from Cycle 1 CHMS.

CHMS participants were chosen using a multi-stage sampling strategy, which included stratification of collection sites by geographic regions and Census Metropolitan Areas (CMA), selection of collection sites according to population size, sampling of dwellings within collection sites with stratification of dwellings by age-groups of inhabitants, and sampling of individuals from dwellings in each age stratum. There was then a random selection of respondents who provided the fasting blood samples in the age group 20-79 (Statistics Canada 2011). People who were living on reserves and other Aboriginal settlements, residents of institutions, full-time members of the Canadian Forces, and those living in certain remote areas with low population densities, were excluded from the CHMS (Statistics Canada 2011). A detailed description of the CHMS is available elsewhere (Statistics Canada 2011).

According to Statistics Canada, the total survey population of Cycle 1 CHMS (2007-2009) included 5,604 individuals from 15 collection sites in five Canadian provinces (New Brunswick, Quebec, Ontario, Alberta, and British Columbia), and was considered representative of 96.3% of the Canadian population (Statistics Canada 2011). A subgroup of 1,696 individuals provided fasting blood samples for DDT and DDE measurement. Participation in CHMS was voluntary

and all 1,696 participants provided informed consent before participation, including consent for the storage and use of their blood samples for future studies (Statistics Canada 2011).

Lung function measures

Lung function parameters considered in this study were FVC (forced vital capacity), FEV_1 (forced expiratory volume in one second), FEV_1/FVC ratio, and $FEF_{25\%-75\%}$ (forced expiratory flow between 25% and 75% of FVC). Health measurement specialists measured lung function among the CHMS participants using a portable spirometer (Koko®, PDS Instrumentation Inc., Louisville, Colorado US). Calibration was performed using a 3 liter syringe. Results were standardized to body temperature, barometric pressure and water saturation (BTPS) (Statistics Canada 2011). American Thoracic Society (ATS) recommendations for performance of spirometry were followed, including obtaining a minimum of 3 acceptable trials from up to 8 attempts based on the ATS definition of within- and between-manoeuvre criteria for usable and acceptable trials (Statistics Canada 2011; Hendrick et al. 2002). The largest value of FVC (or FEV_1) from acceptable trials was used for measuring FVC (or FEV_1) (Statistics Canada 2010b, 2011). The mean flow rate (mL/s) of $FEF_{25\%-75\%}$ from the acceptable trial with the largest sum of FVC and FEV_1 was collected for measuring $FEF_{25\%-75\%}$ (Statistics Canada 2010b, 2011).

***p,p'*-DDT and *p,p'*-DDE concentrations in plasma**

In the current study, concentrations of *p,p'*-DDT and its major metabolite *p,p'*-DDE were measured in blood plasma. All blood samples were centrifuged within two hours and aliquotted within four hours after the blood was drawn (Statistics Canada 2011). Blood samples were then stored frozen at -20°C until concentrations of *p,p'*-DDT and *p,p'*-DDE were measured. Concentrations of *p,p'*-DDT and *p,p'*-DDE in blood plasma (μg/L) were measured using gas chromatography–mass spectrometry (GC-MS) (Health Canada 2010; Statistics Canada 2011).

Detailed laboratory standard operating procedures (SOP) are described at the INSPQ website (INSPQ 2009). Limits of detection (LOD) for *p,p'*-DDT and *p,p'*-DDE were 0.05 μg/L plasma and 0.09 μg/L plasma, respectively (Health Canada 2010). Concentrations of *p,p'*-DDT or *p,p'*-DDE (μg/L plasma) were normalized to total blood lipids and converted to ng/g lipid (Health Canada 2010; Aylward et al. 2010), with total blood lipids calculated as: total lipids (g/L) = 2.27 x 386.65 x cholesterol (mol/L) + 885.45 x triglycerides (mol/L) + 0.623, where 386.65 and 855.45 are the average molecular weights (g/mol) of cholesterol and triglycerides, respectively (Health Canada 2010).

Factors related to lung function

A number of factors that may affect lung function were considered as potential confounders in our analyses, including demographic factors (age, sex, ethnicity, and immigration status), anthropomorphic data (standing height, weight), physical activity (daily energy expenditure) and tobacco smoking status. Factors that were significantly associated with lung function parameters in univariate analyses (p-values<0.1) were considered in the multiple regression models, where the non-significant ones at p=0.05 were removed from the final models.

Information on age, sex, ethnicity and immigration status were collected using a CHMS household questionnaire (Statistics Canada 2010a). Standing height was objectively measured by a fixed stadiometer using standard procedure based on the Canadian Physical Activity, Fitness and Lifestyle Approach (CPAFLA) (CSEP 2003; Statistics Canada 2011). Body mass index (BMI) was calculated using formula weight (kg) / [height (m)]^2. Daily energy expenditure (kcal/kg/day) was derived from the approach used by the Canadian Fitness and Lifestyle Research Institute (http://www.cflri.ca) and National Population Health Survey (NPHS)

(Statistics Canada 2006). Daily Energy Expenditure (DEE, kcal/kg/day) was estimated based on the energy expenditure associated with specific activities (MET = kcal/kg/h) whose frequency and duration were reported on the CHMS household questionnaire (Statistics Canada 2010a). Information from the CHMS household questionnaire regarding the amount and frequency of cigarette smoking was used to classify each participant as a never, former, or current smoker (Statistics Canada 2010a). Pack-years, defined as number of packs of cigarettes smoked per day multiplied by number of years of smoking, were also calculated using detailed information collected on smoking in the CHMS-Cycle 1 (Statistics Canada 2010a). In the pack-years calculation, never smokers and former occasional smokers (< 1 cigarettes smoked / day in the past) were assigned a value of 0 pack-years.

Statistical analyses

Lung function measures FVC, FEV_1, FEV_1/FVC and $FEF_{25\%-75\%}$ were modeled as continuous health outcome variables in the analyses. In regression analyses, plasma *p,p'*-DDT was dichotomized as detectable (>LOD) or not detectable (≤ LOD = 0.05 μg/L plasma) as samples for 90% of participants were ≤ LOD. Plasma *p,p'*-DDE was modeled as a continuous variable because only a small proportion (0.7%) had a concentration ≤ LOD. For participants with *p,p'*-DDE concentrations ≤ LOD, a substitution of 0.5*LOD was used (Rollin et al. 2009). Chi-square test and student-t test were used to examine the difference in the proportion of detecting *p,p'*-DDT in blood and the mean concentrations of *p,p'*-DDE across demographic factors, respectively.

Design weights provided by Statistics Canada to adjust for post-stratification in the multistage sampling, subsampling for the sub-survey, units with no responses, and out of scope responses,

were incorporated in all statistical analyses (Statistics Canada 2011). A re-sampling method using 500 bootstrap weights was applied to calculate the variance of regression coefficient estimates and 95% CIs (Statistics Canada 2011).

Univariate analyses were initially conducted to examine the relationship between risk factors and lung function. Factors that were significantly at p=0.1 were considered in the multiple regression models. In multiple regression models, a purposeful selection method was used in determine the final models, i.e. the known risk factors of lung function, including age, sex, ethnicity, height and smoking, were forced into the final models, and other variables that were non-significant at p=0.05, were excluded from the models.

Associations between lung function parameters and dichotomous *p,p'*-DDT or lipid-normalized *p,p'*-DDE concentrations were estimated by the final multiple linear regression analyses, with lung function as the dependent variable, adjusting for age (continuous), sex, ethnicity (Caucasian or other), height (continuous), smoking status (never, former, current) and daily energy expenditure (continuous). In addition, interactions were not included in final models because none of the interactions between exposures and other covariates on association with lung function outcomes were significant at p=0.05. All statistical analyses were performed with procedures for the complex survey data analysis in STATA (StataCorp LP. 2007. *Stata Statistical Software: Release 12*. College Station, Texas US). This study was approved by the Health Research Ethics Board of the University of Alberta.

Results

Characteristics of the study population

Fasting blood samples for *p,p'*-DDT and *p,p'*-DDE analysis were collected from 1,696 participants aged 20-79 years from five Canadian provinces (Table 1). Among these participants, males and females were almost equally distributed, 22.9% were immigrants, and more than two thirds had Caucasian ethnicity. The study population had an average height of 169.0 cm and average weight of 77.4 kg. In addition, among this study population, 45.8% never smoked, 31.3% were former smokers and 22.9% were current smokers.

p,p'-DDT and *p,p'*-DDE concentrations in the study population

Of 1,696 participants, 10.0% (95% CI: 4.6, 15.4%) had detectable plasma *p,p'*-DDT (Table 2). A significantly higher proportion of non-Caucasians had detectable *p,p'*-DDT compared with Caucasians (25.6% vs. 3.8%), and immigrants were significantly more likely to have detectable *p,p'*-DDT than non-immigrants (34.1% vs. 2.9%).

In this study, more than 99.0% of participants (95% CI: 99.2, 100) had detectable plasma *p,p'*-DDE (Table 2). The average concentration of *p,p'*-DDE was 326.9 ng/g lipid with a median value of 151.9 ng/g lipid (95% CI: 126.9, 191.8) and an interquartile range of (71.5-284.6) ng/g lipid. On average, females had higher plasma *p,p'*-DDE than males (Table 2). Participants aged 60 years and above had a mean concentration of *p,p'*-DDE three times of that for participants aged 20-39 years.

The proportion of participants with detectable *p,p'*-DDT was greater in never smokers than in former and current smokers, and the mean concentration of *p,p'*-DDE was greater in non-smokers than in current and former smokers (Table 2). In addition, participants with detectable

p,p'-DDT had a significantly greater mean *p,p'*-DDE concentration compared to those with no detectable *p,p'*-DDT (1493.3 ng/g lipid; 95% CI: 540.4, 2446.1 vs. 196.1 ng/g lipid; 95% CI: 171.6, 220.6; p =0.012).

Lung function and detectable plasma *p,p'*-DDT

After adjusting for age, sex, ethnicity, height, smoking status and daily energy expenditure, participants with detectable *p,p'*-DDT had a significantly lower mean FVC (diff=311 mL; 95% CI: –492, –130; p=0.003) and FEV_1 (diff=232 mL; 95% CI: –408, –55; p=0.015) than those with non-detectable *p,p'*-DDT (Table 3). *p,p'*-DDT was not associated with the FEV_1/FVC ratio or $FEF_{25\%-75\%}$. Model estimates were similar when adjusted for pack-years instead of smoking status (data not shown).

Lung function and lipid-normalized plasma *p,p'*-DDE concentration

In a multiple linear regression analysis, after adjusting for age, sex, ethnicity, height, smoking status and daily energy expenditure, each 100 ng/g increase in plasma concentration of *p,p'*-DDE was associated with a 18.8 mL reduction in mean FVC (p=0.002) and an 11.8 mL reduction in mean FEV_1 (p=0.013) (Table 3). Plasma *p,p'*-DDE was not associated with the FEV_1/FVC ratio or $FEF_{25\%-75\%}$. Model estimates were similar when adjusting for pack-years instead of categorical smoking status (data not shown).

Discussion

DDT was widely used in agriculture and in the control of malaria and typhus before its use was restricted in the 1970s. Although it has been out of use now for many years, the current results from the CHMS-Cycle 1 (2007-2009) show that almost all Canadian adults aged 20-79 years still had *p,p'*-DDT and/or *p,p'*-DDE detectable in their blood plasma, which is consistent with the

data reported by Health Canada using the same survey data (99.6% and 9.3% had detectable plasma *p,p'*-DDE and *p,p'*-DDT, respectively) (Health Canada 2010). In addition, for participants who had plasma *p,p'*-DDE concentrations less than the LOD also had *p,p'*-DDT non-detectable. Ongoing exposure may arise due to the high persistence of DDT and DDE in the environment (ATSDR 2002). DDT and its metabolites are also highly persistent in the human body, and so our results could also be partially, or wholly, a consequence of exposures some time ago (Longnecker 2005; Wolff et al. 2000). The mean plasma concentration of *p,p'*-DDE reported in this study (152 ng/g lipid adjusted) was lower than that reported from the US National Health And Nutrition Examination Survey (NHANES III, 1999-2004, 238-260 ng/g lipid adjusted) (CDC 2009; EPA 2008), indicating a lower exposure to DDT and its related compounds in Canada than in the US.

Although there have been a number of studies suggesting an adverse effect of pesticides on pulmonary function (Beseler and Stallones 2009; Chakraborty et al. 2009; Fareed et al. 2013; Hernandez et al. 2008; Mekonnen and Agonafir 2004; Peiris-John et al. 2005; Rastogi et al. 1989; Salameh et al. 2005; Zuskin et al. 2008), most have lacked information on the specific types of pesticides used (Beseler and Stallones 2009; Mekonnen and Agonafir 2004; Salameh et al. 2005; Zuskin et al. 2008), while others focused on pesticides other than DDT, such as organophosphate or carbamate insecticides (Chakraborty et al. 2009; Fareed et al. 2013; Peiris-John et al. 2005).

We estimated that among a representative sample of Canadian adults aged 20-79 years, participants with detectable plasma *p,p'*-DDT had significantly lower mean FVC and FEV_1 than those with plasma *p,p'*-DDT $\leq$ LOD. The estimated magnitude of FVC and FEV_1 reduction associated with DDT exposure reported in this study (310.7 mL and 231.8 mL, respectively) is similar to the natural decline of lung function (30mL/year in FVC and 20-30 mL/year in FEV_1)

for a healthy non-smoker adults over a 10 years period (Burrows et al. 1983; Peat et al. 1990). In addition, lipid normalized plasma *p,p'*-DDE concentrations were negatively associated with FVC and FEV_1 when modeled as a continuous variable. To the best of our knowledge, this study is the first population-based investigation of the association of DDT and its metabolite DDE with lung function among Canadian adults.

Several studies in the literature have also reported that exposures to other organochlorine pesticides are associated with reductions in lung function. For example, a study among agricultural pesticide sprayers in Spain reported that exposures to endosulfan were negatively associated with FEV_1 and $FEF_{25\%-75\%}$ (Hernandez et al. 2008). Another study among pesticide spraying workers in India reported that a restrictive type of impairment of lung function was associated with exposures to unspecified organochlorine insecticides (Rastogi et al. 1989), which is consistent with the negative association between DDT/DDE and lung function estimated in the present study.

Exposure to DDT has also been associated with the prevalence of respiratory diseases. Hoppin et al in the Agricultural Health Study reported that DDT exposures were associated with nonatopic asthma among male farmers (Hoppin et al. 2009) and atopic asthma among female farmers (Hoppin et al. 2008). In addition, Hoppin et al. reported that the lifetime number of days of occupational application of DDT in agriculture was significantly associated with higher prevalence of chronic bronchitis (Hoppin et al. 2007). Another study using the same dataset found that the prevalence of chronic bronchitis among female non-smoking farmers was significantly associated with the use of DDT (Valcin et al. 2007).

DDT and related compounds are neurotoxicants that bind to voltage-gated sodium channels to prevent their closure, which leads to increased sodium influx and repeated firing of neurons (Keifer and Firestone 2007). In addition, physiological studies of animal models have shown that sodium influx and subsequent depolarization of neurons in general can cause contractile responses of airway smooth muscles (Souhrada et al. 1988; Souhrada and Souhrada 1989). Moreover, DDT and its metabolite DDE have been shown to be able to activate stress-response signalling *in vitro*, including ERK-MAPK, JNK and NFkB signalling pathways, which result in an intracellular release of calcium (Abdollahi et al. 2004; Androutsopoulos et al. 2012). Calcium release into cytoplasm has also been shown to lead to contraction of airway smooth muscles in studies of airway smooth muscle cells and animal model rat (Berridge 2008; Menshikova et al. 1995; Sakai et al. 2013; Tomasic et al. 1992). Airway narrowing in response to DDT/DDE exposures would be consistent with the negative associations between exposures and lung function in our study population.

Changes in immune system parameters and markers of immune function have been associated with DDT/DDE exposures in observational (Daniel et al. 2001, 2002; Vine et al. 2001), which suggests that exposure might contribute to impaired lung function by increasing airway sensitization or inflammation.

Previous studies of pesticides and respiratory outcomes often used questionnaire-based approaches or job titles to classify pesticide exposures (Ejigu and Mekonnen 2005; Hernandez et al. 2008; Mekonnen and Agonafir 2004; Salameh et al. 2005; Zuskin et al. 2008), both of which are liable to errors and bias. In this study, we used a biomonitoring approach to measure DDT/DDE exposures, i.e. objectively testing the concentration of *p,p'*-DDT/DDE in blood plasma (Kapka-Skrzypczak et al. 2011). The concentration of pesticide measured by

biomonitoring method is likely a good estimate of actual body burden arising from exposures to bioaccumulative chemicals, and hence is a good alternative for measuring cumulative exposures. For DDT and DDE, this is particularly so because of their long half-life in the human body, which makes them a good marker of past or cumulative exposure in research and environmental surveillance projects (CDC 2005; Reigart and Roberts 1999).

There are several limitations in this study. Firstly, the CHMS survey was not fully representative of the Canadian population. Aboriginal people living on reserves and Aboriginal settlements, people living in remote areas, residents of institutions and full-time members of the Canadian Forces were not included in the CHMS (Statistics Canada 2011). However, the excluded populations in the CHMS represent less than 4% of the total Canadian populations (Statistics Canada 2011). Secondly, in the current study, only one of the 13 isomers of the insecticide DDT (Kroschwitz et al. 1995), *p,p'*-DDT and its metabolite *p,p'*-DDE, were measured. The rest of the 12 isomers might have been present in blood samples and were not monitored (Statistics Canada 2011). Thirdly, in the current study, associations between DDT/DDE and lung function parameters were characterized among participants aged 20-79 years. Potential effects of DDT and DDE on respiratory health may also be critical for subjects with younger ages. For example, birth cohort studies in Spain suggested that perinatal exposure to DDT was positively associated with asthma prevalence and persistent wheezing in children (Sunyer et al. 2005; Sunyer et al. 2006). In addition, associations between respiratory tract infection and DDT/DDE exposures have also been reported among young children (Dallaire et al. 2004; Sunyer et al. 2010). A future study of the effect of DDT/DDE on lung function among children and youth is necessary. Lastly, due to the cross-sectional nature of the CHMS, the temporal sequence between changes in lung function and DDT exposures is not clear. In addition, analyses of DDT using a dichotomous

exposure, due to the large proportion of participants having no detectable level of DDT, may lead to a potential bias due to uncontrolled confounding or misclassification.

Conclusions

Although a worldwide ban of DDT for agricultural use has been in place since 2004 when the Stockholm Convention classified DDT as persistent organic pollutant (POP), DDT is still currently produced and used in many countries, including China, India, South Africa, Ethiopia and North Korea (UNEP 2010). Our results show that *p,p'*-DDE, the metabolite of insecticide DDT was detectable in almost all blood samples of Canadian adults aged 20-79 years, indicating that exposure to DDT is still a health concern, despite a ban in Canada many decades ago. Issues related to the health impact of DDT have been raised since Rachel Carson's well known book 'Silent Spring' was published in the early 1960s (Carson 1962). However, there is still limited evidence for an effect of DDT on respiratory health. Our study is the first population-based study of Canadian adults demonstrating that plasma DDT, and its metabolite DDE, were negatively associated with two measures of lung function, specifically FVC and FEV_1.

References

Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. 2004. Pesticides and oxidative stress: A review. Med Sci Monit 10:RA141-147.

Anderson HA. 1985. Utilization of adipose tissue biopsy in characterizing human halogenated hydrocarbon exposure. Environ Health Perspect 60:127-131.

Androutsopoulos VP, Hernandez AF, Liesivuori J, Tsatsakis AM. 2012. A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides. Toxicology 307: 89-94.

ATSDR. 2002. Toxicological profile for DDT, DDE, and DDD. Agency for Toxic Substances and Disease Registry. Available: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=81&tid=20 [accessed 08 December 2014].

Attaran A, Maharaj R. 2000. Ethical debate: Doctoring malaria, badly: The global campaign to ban DDT. BMJ 321:1403-1405.

Aylward LL, Hays SM, Gagne M, Nong A, Krishnan K. 2010. Biomonitoring equivalents for hexachlorobenzene. Regul Toxicol Pharmacol 58:25-32.

Bayen S, Giusti P, Lee HK, Barlow PJ, Obard JP. 2005. Bioaccumulation of DDT pesticide in cultured asian seabass following dietary exposure. J Toxicol Environ Health A 68:51-65.

Beard J, Sladden T, Morgan G, Berry G, Brooks L, McMichael A. 2003. Health impacts of pesticide exposure in a cohort of outdoor workers. Environ Health Perspect 111:724-730.

Beard J. 2006. DDT and human health. Sci Total Environ 355:78-89.

Berridge MJ. 2008. Smooth muscle cell calcium activation mechanisms. J Physiol 586:5047-5061.

Beseler CL, Stallones L. 2009. Pesticide poisoning and respiratory disorders in colorado farm residents. J Agric Saf Health 15:327-334.

Burrows B, Cline MG, Knudson RJ, Taussig LM, Lebowitz MD. 1983. A descriptive analysis of the growth and decline of the FVC and FEV_1. Chest 83:717-724.

Carson R. 1962. Silent Spring. 1st ed. Boston:Houghton Mifflin.

CDC. 2005. Third national report on human exposure to environmental chemicals. Centers for Disease Control and Prevention. Atlanta, GA.

CDC. 2009. Fourth national report on human exposure to environmental chemicals. Centers for Disease Control and Prevention. Available: http://www.cdc.gov/exposurereport/ [accessed 08 December 2014].

Chakraborty S, Mukherjee S, Roychoudhury S, Siddique S, Lahiri T, Ray MR. 2009. Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India. J Occup Health 51:488-497.

Chen Z, Maartens F, Vega H, Kunene S, Gumede J, Krieger RI. 2009. 2,2-bis(4-chlorophenyl)acetic acid (DDA), a water-soluble urine biomarker of DDT metabolism in humans. Int J Toxicol 28:528-533.

Corsini E, Liesivuori J, Vergieva T, Van Loveren H, Colosio C. 2008. Effects of pesticide exposure on the human immune system. Hum Exp Toxicol 27:671-680.

CSEP. 2003. Canadian physical activity, fitness and lifestyle approach. 3rd ed. Canadian Society for Exercise Physiology. Ottawa.

Dallaire F, Dewailly E, Muckle G, Vezina C, Jacobson SW, Jacobson JL, et al. 2004. Acute infections and environmental exposure to organochlorines in Inuit infants from Nunavik. Environ Health Perspect 112:1359-1365.

Daniel V, Huber W, Bauer K, Suesal C, Conradt C, Opelz G. 2001. Associations of blood levels of PCB, HCHs, and HCB with numbers of lymphocyte subpopulations, in vitro lymphocyte response, plasma cytokine levels, and immunoglobulin autoantibodies. Environ Health Perspect 109:173-178.

Daniel V, Huber W, Bauer K, Suesal C, Conradt C, Opelz G. 2002. Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyldichloroethylene (DDE) 4.4 blood levels with plasma IL-4. Arch Environ Health 57:541-547.

De Coster S, van Larebeke N. 2012. Endocrine-disrupting chemicals: Associated disorders and mechanisms of action. J Environ Public Health 2012:713696.

Ejigu D, Mekonnen Y. 2005. Pesticide use on agricultural fields and health problems in various activities. East Afr Med J 82:427-432.

EPA. 2008. U.S. EPA's 2008 report on the environment (final report). Environment protection agency. Available: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=190806 [accessed 08 December 2014].

Eskenazi B, Chevrier J, Rosas LG, Anderson HA, Bornman MS, Bouwman H, et al. 2009. The pine river statement: Human health consequences of DDT use. Environ Health Perspect 117:1359-1367.

Fareed M, Pathak MK, Bihari V, Kamal R, Srivastava AK, Kesavachandran CN. 2013. Adverse respiratory health and hematological alterations among agricultural workers occupationally exposed to organophosphate pesticides: A cross-sectional study in north India. PLoS One 8:e69755.

Health Canada. 2010. Report on human biomonitoring of environmental chemicals in Canada-results of the Canadian Health Measures Survey Cycle 1 (2007-2009). Available: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms/index-eng.php. [accessed 08 December 2014].

Hendrick DJ, Burge SP, Beckett WS, Churg A. 2002. Occupational disorders of the lung: Recognition, management, and prevention. 1st ed. London,WB Saunders.

Hernandez AF, Casado I, Pena G, Gil F, Villanueva E, Pla A. 2008. Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects. Inhal Toxicol 20:839-849.

Hoppin JA, Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, et al. 2007. Pesticide use and chronic bronchitis among farmers in the Agricultural Health Study. Am J Ind Med 50:969-979.

Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Alavanja MC, et al. 2008. Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. Am J Respir Crit Care Med 177:11-18.

Hoppin JA, Umbach DM, London SJ, Henneberger PK, Kullman GJ, Coble J, et al. 2009. Pesticide use and adult-onset asthma among male farmers in the Agricultural Health Study. Eur Respir J 34:1296-1303.

INSPQ. 2009. Analytical method for the determination of polychlorinated biphenyl congeners, polybrominated congeners, toxaphene congeners, and organochlorinated pesticides in plasma by GC-MS (e-446), condensed version. Laboratoire de Toxicologie, Institut National de Santé Publique du Québec.

Kapka-Skrzypczak L, Cyranka M, Skrzypczak M, Kruszewski M. 2011. Biomonitoring and biomarkers of organophosphate pesticides exposure - state of the art. Ann Agric Environ Med 18:294-303.

Keifer MC, Firestone J. 2007. Neurotoxicity of pesticides. J Agromedicine 12:17-25.

Kroschwitz J, Howe-Grant M, Metcalf R. 1995. Kirk-Othmer encyclopedia of chemical technology. New York, NY, John Wiley and Sons, Inc.

Longnecker MP. 2005. Invited commentary: Why DDT matters now. Am J Epidemiol 162:726-728.

Mekonnen Y, Agonafir T. 2004. Lung function and respiratory symptoms of pesticide sprayers in state farms of Ethiopia. Ethiop Med J 42:261-266.

Menshikova EV, Ritov VB, Shvedova AA, Elsayed N, Karol MH, Kagan VE. 1995. Pulmonary microsomes contain a Ca(2+)-transport system sensitive to oxidative stress. Biochim Biophys Acta 1228:165-174.

Peat JK, Woolcock AJ, Cullen K. 1990. Decline of lung function and development of chronic airflow limitation: A longitudinal study of non-smokers and smokers in Busselton, Western Australia. Thorax 45:32-37.

Peiris-John RJ, Ruberu DK, Wickremasinghe AR, van-der-Hoek W. 2005. Low-level exposure to organophosphate pesticides leads to restrictive lung dysfunction. Respir Med 99:1319-1324.

Perez-Maldonado IN, Trejo A, Ruepert C, Jovel Rdel C, Mendez MP, Ferrari M, et al. 2010. Assessment of DDT levels in selected environmental media and biological samples from Mexico and Central America. Chemosphere 78:1244-1249.

Rastogi SK, Gupta BN, Husain T, Mathur N, Garg N. 1989. Study of respiratory impairment among pesticide sprayers in mango plantations. Am J Ind Med 16:529-538.

Reigart JR, Roberts JR. 1999. Recognition and management of pesticide poisonings. Available: http://npic.orst.edu/rmpp.htm [accessed 08 December 2014].

Rollin HB, Sandanger TM, Hansen L, Channa K, Odland JO. 2009. Concentration of selected persistent organic pollutants in blood from delivering women in South Africa. Sci Total Environ 408:146-152.

Sakai H, Watanabe Y, Honda M, Tsuiki R, Ueda Y, Nagai Y, et al. 2013. Involvement of the Tyr kinase/JNK pathway in carbachol-induced bronchial smooth muscle contraction in the rat. Anesthesiology 118:1076-1085.

Salameh P, Waked M, Baldi I, Brochard P. 2005. Spirometric changes following the use of pesticides. East Mediterr Health J 11:126-136.

Smith D. 1999. Worldwide trends in DDT levels in human breast milk. Int J Epidemiol 28:179-188.

Souhrada M, Souhrada MH, Souhrada JF. 1988. The inhibition of sodium influx attenuates airway response to a specific antigen challenge. Br J Pharmacol 93:884-892.

Souhrada M, Souhrada JF. 1989. Sodium and calcium influx induced by phorbol esters in airway smooth muscle cells. Am Rev Respir Dis 139:927-932.

Statistics Canada. 2006. National Population Health Survey - household component cycle 6 (2004/2005): Derived variables documentation. Available: http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&SurvId=1824&InstaId=7801&SDDS=3225 [accessed 08 December 2014].

Statistics Canada. 2010a. Canadian Health Measures Survey (Cycle 1) - household questionnaire Available: http://www23.statcan.gc.ca/imdb/pIX.pl?Function=showStaticArchiveHTML&a=1&fl=http://www23.statcan.gc.ca/imdb-bmdi/instrument/5071_Q2_V1-eng.htm&Item_Id=64294[accessed 08 December 2014].

Statistics Canada. 2010b. Canadian Health Measures Survey (CHMS) Cycle 1: Data dictionary.

Statistics Canada. 2011. Canadian Health Measures Survey (CHMS) data user guide: Cycle 1. Available: http://www23.statcan.gc.ca/imdb-bmdi/pub/document/5071_D2_T1_V1-eng.htm [accessed 08 December 2014].

Sunyer J, Torrent M, Munoz-Ortiz L, Ribas-Fito N, Carrizo D, Grimalt J, et al. 2005. Prenatal dichlorodiphenyldichloroethylene (DDE) and asthma in children. Environ Health Perspect 113:1787-1790.

Sunyer J, Torrent M, Garcia-Esteban R, Ribas-Fito N, Carrizo D, Romieu I, et al. 2006. Early exposure to dichlorodiphenyldichloroethylene, breastfeeding and asthma at age six. Clin Exp Allergy 36:1236-1241.

Sunyer J, Garcia-Esteban R, Alvarez M, Guxens M, Goni F, Basterrechea M, et al. 2010. DDE in mothers' blood during pregnancy and lower respiratory tract infections in their infants. Epidemiology 21:729-735.

Tomasic M, Boyle JP, Worley JF, 3rd, Kotlikoff MI. 1992. Contractile agonists activate voltage-dependent calcium channels in airway smooth muscle cells. Am J Physiol 263:C106-113.

UNEP. 2010. Report of the third DDT expert group meeting-Stochholm Convention. Available: http://chm.pops.int/Implementation/DDT/Meetings/DDTEG32010/tabid/1108/mctl/ViewDetails/EventModID/1421/EventID/116/xmid/4037/Default.aspx [accessed 08 December 2014].

Valcin M, Henneberger PK, Kullman GJ, Umbach DM, London SJ, Alavanja MC, et al. 2007. Chronic bronchitis among nonsmoking farm women in the Agricultural Health Study. J Occup Environ Med 49:574-583.

Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK, et al. 2001. Plasma 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) levels and immune response. Am J Epidemiol 153:53-63.

WHO. 1979. DDT and its derivatives. World Health Organization. Geneva.

Wolff MS, Zeleniuch-Jacquotte A, Dubin N, Toniolo P. 2000. Risk of breast cancer and organochlorine exposure. Cancer Epidemiol Biomarkers Prev 9:271-277.

Ye M, Beach J, Martin JW, Senthilselvan A. 2013. Occupational pesticide exposures and respiratory health. Int J Environ Res Public Health 10:6442-6471.

Zuskin E, Mustajbegovic J, Schachter EN, Kern J, Deckovic-Vukres V, Trosic I, et al. 2008. Respiratory function in pesticide workers. J Occup Environ Med 50:1299-1305.

Table 1. Characteristics of the study population.

Characteristics	% or mean* (95% CI or ± S.E.†)
Total sample (N=1,696)	
Age (%, years)	
20-39	37.9 (37.9, 37.9)
40-59	41.3 (41.3, 41.3)
60-79	20.7 (20.7, 20.7)
Sex (%)	
Female	50.6 (50.4, 50.9)
Male	49.4 (49.1, 49.6)
Height (mean, cm)	169.0 ± 0.4
Weight (mean, kg)	77.4 ± 0.9
Ethnicity (%)	
Caucasian	71.4 (62.7, 80.1)
Others	28.6 (19.9, 37.3)
Immigrant (%)	
No	77.1 (66.6, 87.6)
Yes	22.9 (12.4, 33.4)
Province of residence (%)	
New Brunswick	7.2 (0, 22.1)
Quebec	23.8 (8.9, 38.6)
Ontario	38.9 (38.9, 38.9)
Alberta	16.6 (16.6, 16.6)
British Columbia	13.6 (13.6, 13.6)
Smoking status (%)	
Never	45.8 (42.0, 49.6)
Former smoker	31.3 (28.0, 34.6)
Current smoker	22.9 (20.4, 25.4)

*Survey design weights were used in calculating percentages and mean values of the study population, a representative sample of the Canadian adults.

†Survey design weights and 500 bootstrap weights were included in calculating the standard errors (S.E.) and 95% CI.

Table 2. Plasma *p,p'*-DDT and *p,p'*-DDE among the study population by demographic factors and smoking status[*†].

	***p,p'*-DDT**		***p,p'*-DDE**	
Characteristics	**% ≥LOD* (95% CI[†])**	***p*-value**	**Mean* (ng/g, 95% CI[†])**	***p*-value**
Total sample	10.0 (4.6, 15.4)		326.9 (210.7, 443.0)	
Age (years)				
20-39	9.1 (3.9, 14.2)		198.6 (115.1, 282.1)	
40-59	9.5 (2.6, 16.4)	0.53	281.9 (188.8, 374.9)	0.023
60-79	12.8 (7.4, 18.2)	0.10	648.0 (280.4, 1015.6)	0.014
Sex				
Female	11.3 (5.8, 16.8)		418.7 (235.0, 602.5)	
Male	8.7 (2.7, 14.8)	0.23	235.4 (169.0, 301.8)	0.021
Ethnicity				
Caucasian	3.8 (2.5, 5.1)		197.6 (171.2, 224.1)	
Others	25.6 (13.9, 37.3)	<0.0001	648.4 (305.4, 991.5)	0.015
Immigrant				
No	2.9 (1.4, 4.4)		173.9 (153.9, 193.8)	
Yes	34.1 (19.5, 48.7)	<0.0001	650.1 (452.6, 847.7)	<0.0001
Smoking status				
Never	15.3 (6.6, 24.1)		432.4 (217.7, 647.0)	
Former smoker	7.1 (2.6, 11.6)	0.056	273.8 (221.6, 326.0)	0.060
Current smoker	3.1 (0.5, 5.7)	0.003	183.0 (141.8, 224.2)	0.033

[*]Mean concentrations of *p,p'*-DDT were lower than LOD as a higher proportion of participants had no *p,p'*-DDT detectable in plasma. Mean concentrations of *p,p'*-DDE were calculated among all participants and for participants with concentrations less than LOD (less than 1.0%), 0.5*LOD was used.

[†]Survey design weights and 500 bootstrap weights were used in calculating percentages, mean values and the 95% confidence intervals.

Table 3. Results from the multiple linear regression of lung function parameters and plasma *p,p'*-DDT and *p,p'*-DDE.*

	FVC (mL)		FEV_1 (mL)		FEV_1/FVC (%)		$FEF_{25-75\%}$ (mL/s)	
Exposure	**Beta (95% CI)**	***p*-value**	**Beta (95% CI)**	***p*-value**	**Beta (95% CI)**	***p*-value**	**Beta (95% CI)**	***p*-value**
***p,p'*-DDT**								
<LOD	0		0		0		0	
≥LOD	-310.7 (-491.8, -129.6)	0.003	-231.8 (-408.3, -55.3)	0.015	0.08 (-1.71, 1.87)	0.925	-98.6 (-435.7, 238.5)	0.533
***p,p'*-DDE** per 100 ng/g lipid	-18.8 (-28.7, -8.9)	0.002	-11.8 (-20.6, -3.1)	0.013	0.09 (-0.11, 0.28)	0.363	-2.2 (-27.3, 22.9)	0.850

*Beta coefficients were obtained after adjusting for age, sex, ethnicity (Caucasian or other), height and smoking status (never, former and current smokers) and daily energy expenditure. Survey design weights and 500 bootstrap weights were included in calculating β coefficients, 95% confidence intervals and variance estimation. Beta=β coefficients.